

WHAT IS CLAIMED IS:

1. A capture probe suitable for use with a method for isolating miRNAs, the capture probe comprising:

a) a first adapter segment having a first adapter segment sequence, the first adapter segment comprising a 3' end and a 5' end;

b) a second adapter segment having a second adapter segment sequence, the second adapter segment comprising a 3' end and a 5' end; and

c) an miRNA binding segment having an miRNA binding segment sequence;

where the miRNA binding segment is substantially complementary to, and capable of hybridizing to, one or more than one miRNA of interest by Watson-Crick base pairing;

where the 5' end of the first adapter segment is connected to the 3' end of the miRNA binding segment; and

where the 3' end of the second adapter segment is connected to the 5' end of the miRNA binding segment.

2. The capture probe of claim 1, comprising a substance selected from the group consisting of one or more than one type of polynucleotide, one or more than one type of polynucleotide analog, and a combination of one or more than one type of polynucleotide and polynucleotide analog.

3. A set of capture probes, where each of the capture probes of the set of capture probes is a capture probe according to claim 1;

where each of the capture probes comprises identical first adapter segment sequences;

where each of the capture probes of the set of capture probes comprises identical miRNA binding segment sequences; and

where each of the capture probes of the set of capture probes comprises identical second adapter segment sequences.

4. A set of capture probes, where each of the capture probes is a capture probe according to claim 1; and

where the set comprises at least one capture probe comprising an miRNA binding segment that is substantially complementary to, and capable of hybridizing to, each miRNA listed in a single public database.

5. A set of capture probes, where each of the capture probes is a capture probe according to claim 1;

where the set comprises a first capture probe and a second capture probe;

where the first capture probe and the second capture probe have identical first adapter segment sequences;

where the first capture probe and the second capture probe have identical miRNA binding segment sequences; and

where the first capture probe has a second adapter segment sequence that is different from the second adapter segment sequence of the second capture probe.

6. A set of capture probes, where each of the capture probes is a capture probe according to claim 1;

where the set comprises a first capture probe and a second capture probe;

where the first capture probe and the second capture probe have identical first adapter segment sequences;

where the first capture probe and the second capture probe have identical second adapter segment sequences; and

where the first capture probe has an miRNA binding segment sequence that is different from the miRNA binding segment sequence of the second capture probe.

7. A set of capture probes, where each of the capture probes is a capture probe according to claim 1;

where the set comprises a first capture probe and a second capture probe;

where the first capture probe and the second capture probe have identical miRNA binding segment sequences;

where the first capture probe and the second capture probe have identical second adapter segment sequences; and

where the first capture probe has a first adapter segment sequence that is different from the first adapter segment sequence of the second capture probe.

8. A set of capture probes, where each of the capture probes is a capture probe according to claim 1;

where the set comprises a first capture probe and a second capture probe;

where the first capture probe and the second capture probe have identical first adapter segment sequences;

where the first capture probe has an miRNA binding segment sequence that is different from the miRNA binding segment sequence of the second capture probe; and

where the first capture probe has a second adapter segment sequence that is different from the second adapter segment sequence of the second capture probe.

9. A set of capture probes, where each of the capture probes is a capture probe according to claim 1;

5 where the set comprises a first capture probe and a second capture probe;

 where the first capture probe and the second capture probe have identical miRNA binding segment sequences;

 where the first capture probe has a first adapter segment sequence that is different from the first adapter segment sequence of the second capture probe; and

10 where the first capture probe has a second adapter segment sequence that is different from the second adapter segment sequence of the second capture probe.

10. A set of capture probes, where each of the capture probes is a capture probe according to claim 1;

 where the set comprises a first capture probe and a second capture probe;

15 where the first capture probe and the second capture probe have identical second adapter segment sequences;

 where the first capture probe has a first adapter segment sequence that is different from the first adapter segment sequence of the second capture probe; and

20 where the first capture probe has an miRNA binding segment sequence that is different from the miRNA binding segment sequence of the second capture probe.

11. A set of capture probes, where each of the capture probes is a capture probe according to claim 1;

 where the set comprises a first capture probe and a second capture probe;

25 where the first capture probe has a first adapter segment sequence that is different from the first adapter segment sequence of the second capture probe;

 where the first capture probe has an miRNA binding segment sequence that is different from the miRNA binding segment sequence of the second capture probe; and

 where the first capture probe has a second adapter segment sequence that is different from the second adapter segment sequence of the second capture probe.

30 12. The capture probe of claim 1, where the first adapter segment, or the second adapter segment, or both the first adapter segment and the second adapter segment are between 6 and 16 residues.

13. The capture probe of claim 1, where the first adapter segment, or the second adapter segment, or both the first adapter segment and the second adapter segment further comprise a sequence that is a polynucleotide synthesis promoter motif for a polynucleotide polymerase, or that is complementary to a polynucleotide synthesis promoter motif for a polynucleotide polymerase.

14. The capture probe of claim 13, where the polynucleotide synthesis promoter motif is a motif for a polynucleotide synthesis promoter selected from the group consisting of T7, SP6, a T3 DNA dependent RNA polymerase, a type 2 RNA polymerase of *E. coli* and single stranded DNA dependent N4 RNA polymerase.

15. The capture probe of claim 1, where the first adapter segment, or the second adapter segment, or both the first adapter segment and the second adapter segment further comprise a restriction site motif.

16. The capture probe of claim 15, where the restriction site motif is acted upon by a restriction enzyme selected from the group consisting of Not I, Xho I, Xma I and Nhe I.

17. The capture probe of claim 1, where the first adapter segment, or the second adapter segment, or both the first adapter segment and the second adapter segment further comprise a solid phase binding group to immobilize the capture probe to a solid phase.

18. The capture probe of claim 17, where the solid phase binding group is at or near the 3' end of the first adapter segment.

19. The capture probe of claim 17, where the solid phase binding group is at or near the 5' end of the second adapter segment.

20. The capture probe of claim 17, where the solid phase binding group immobilizes the capture probe to the solid phase covalently.

21. The capture probe of claim 17, where the solid phase binding group immobilizes the capture probe to the solid phase non-covalently.

22. The capture probe of claim 17, where the solid phase binding group comprises biotin or an analog of biotin.

23. The capture probe of claim 1, where the miRNA binding segment consists of 18 or 19 or 20 or 21 or 22 or 23 or 24 residues selected from the group consisting of DNA, RNA, chimeric DNA/RNA, DNA analogs and RNA analogs.

24. The capture probe of claim 1, where the miRNA of interest that the miRNA binding segment is substantially complementary to, and capable of hybridizing to, is selected

from a public database.

25. The capture probe of claim 1, where the miRNA of interest is a eucaryotic miRNA.

26. The capture probe of claim 1, where the miRNA of interest is a primate miRNA.

5 27. The capture probe of claim 1, where the miRNA of interest is a human miRNA.

28. The capture probe of claim 1, where the miRNA binding segment is exactly the complement to the miRNA of interest in both length and sequence.

10 29. The capture probe of claim 1, where the miRNA binding segment is more than 90% complementary to a segment of the miRNA of interest of the same length as the miRNA of interest sequence.

30. The capture probe of claim 1, where the miRNA binding segment is more than 80% complementary to a segment of the miRNA of interest of the same length as the miRNA of interest sequence.

15 31. The capture probe of claim 1, where the first adapter segment has a first adapter segment sequence according to SEQ ID NO:1.

32. The capture probe of claim 1, where the second adapter segment has a second adapter segment sequence according to SEQ ID NO:2.

33. A method for isolating an miRNA of interest from a sample comprising the miRNA of interest; the method comprising:

20 a) providing a sample comprising the miRNA of interest;

b) providing a capture probe according to claim 1;

c) providing a first linker and a second linker;

d) combining the sample, the capture probe, the first linker and the second linker;

25 e) allowing the first linker to hybridize with the first adapter segment, the miRNA of interest to hybridize with the miRNA binding segment, and the second linker to hybridize with the second adapter segment;

30 f) ligating the 3' end of the first linker that is hybridized to the first adapter segment to the 5' end of the miRNA of interest that is hybridized to the miRNA binding segment, and ligating the 3' end of the miRNA of interest that is hybridized to the miRNA binding segment to the 5' end of the second linker that is hybridized to the second adapter segment, thereby producing a complex defined as a strand of first linker, miRNA of interest and second linker that have been ligated together (ligated first linker-miRNA of interest-second linker) and that

is hybridized to the capture probe; and

g) dehybridizing the capture probe from the strand of the ligated first linker-miRNA of interest-second linker;

where the miRNA of interest has an miRNA of interest sequence, and comprises a 3' end and a 5' end;

where the miRNA of interest is substantially complementary to, and capable of hybridizing to, the miRNA binding segment of the capture probe by Watson-Crick base pairing;

where the first linker has a first linker sequence, and comprises a 3' end and a 5' end;

where the first linker is substantially complementary to, and capable of hybridizing to, the first adapter segment of the capture probe by Watson-Crick base pairing;

where the second linker has a second linker sequence, and comprises a 3' end and a 5' end; and

where the second linker is substantially complementary to, and capable of hybridizing to, the second adapter segment of the capture probe by Watson-Crick base pairing.

34. The method of claim 33, where the sample further comprises one or more than one substance that is chemically related to the miRNA of interest selected from the group consisting of an RNA other than a miRNA and a DNA.

35. The method of claim 33, where the sample is from a eukaryote.

36. The method of claim 33, where the sample is from a primate.

37. The method of claim 33, where the sample is from a human.

38. The method of claim 33, where the sample comprises a tissue or fluid selected from the group consisting of blood, brain, heart, intestine, liver, lung, pancreas, muscle, a leaf, a flower, a plant root and a plant stem.

39. The method of claim 33, where the miRNA of interest consists of 18 or 19 or 20 or 21 or 22 or 23 or 24 RNA residues.

40. The method of claim 33, where the miRNA of interest is listed in a public database.

41. The method of claim 33, where the sample provided comprises a plurality of miRNAs of interest; and

where each of the plurality of miRNAs of interest has miRNA of interest sequences that are identical to one another.

42. The method of claim 33, where the sample provided comprises a plurality of miRNAs of interest comprising a first miRNA of interest having a first miRNA of interest sequence, and a second miRNA of interest having a second miRNA of interest sequence; and
5 where the first miRNA of interest sequence is different from the second miRNA of interest sequence.

43. The method of claim 33, where the sample provided comprises a plurality of miRNAs of interest comprising a first miRNA of interest having a first miRNA of interest sequence, a second miRNA of interest having a second miRNA of interest sequence, and a third miRNA of interest having a third miRNA of interest sequence;

10 where the first miRNA of interest sequence is different from the second miRNA of interest sequence;

where the first miRNA of interest sequence is different from the third miRNA of interest sequence; and

15 where second miRNA of interest sequence is different from the third miRNA of interest sequence.

44. The method of claim 33, further comprising isolating the total RNA from the sample after providing the sample.

45. The method of claim 33, where the capture probe provided is a set of capture probes;

20 where each of the capture probes comprises identical first adapter segment sequences;

where each of the capture probes of the set of capture probes comprises identical miRNA binding segment sequences; and

where each of the capture probes of the set of capture probes comprises identical second adapter segment sequences.

25 46. The method of claim 33, where the capture probe provided is a set of capture probes;

where the set comprises at least one capture probe comprising an miRNA binding segment that is substantially complementary to, and capable of hybridizing to, each miRNA listed in a single public database.

30 47. The method of claim 33, where the capture probe provided is a set of capture probes;

where the set comprises a first capture probe and a second capture probe;

where the first capture probe and the second capture probe have identical first adapter segment sequences;

where the first capture probe and the second capture probe have identical miRNA binding segment sequences; and

5 where the first capture probe has a second adapter segment sequence that is different from the second adapter segment sequence of the second capture probe.

48. The method of claim 33, where the capture probe provided is a set of capture probes;

where the set comprises a first capture probe and a second capture probe;

10 where the first capture probe and the second capture probe have identical first adapter segment sequences;

where the first capture probe and the second capture probe have identical second adapter segment sequences; and

15 where the first capture probe has an miRNA binding segment sequence that is different from the miRNA binding segment sequence of the second capture probe.

49. The method of claim 33, where the capture probe provided is a set of capture probes;

where the set comprises a first capture probe and a second capture probe;

20 where the first capture probe and the second capture probe have identical miRNA binding segment sequences;

where the first capture probe and the second capture probe have identical second adapter segment sequences; and

where the first capture probe has a first adapter segment sequence that is different from the first adapter segment sequence of the second capture probe.

25 50. The method of claim 33, where the capture probe provided is a set of capture probes;

where the set comprises a first capture probe and a second capture probe;

where the first capture probe and the second capture probe have identical first adapter segment sequences;

30 where the first capture probe has an miRNA binding segment sequence that is different from the miRNA binding segment sequence of the second capture probe; and

where the first capture probe has a second adapter segment sequence that is different

from the second adapter segment sequence of the second capture probe.

51. The method of claim 33, where the capture probe provided is a set of capture probes;

where the set comprises a first capture probe and a second capture probe;

5 where the first capture probe and the second capture probe have identical miRNA binding segment sequences;

where the first capture probe has a first adapter segment sequence that is different from the first adapter segment sequence of the second capture probe; and

10 where the first capture probe has a second adapter segment sequence that is different from the second adapter segment sequence of the second capture probe.

52. The method of claim 33, where the capture probe provided is a set of capture probes;

where the set comprises a first capture probe and a second capture probe;

15 where the first capture probe and the second capture probe have identical second adapter segment sequences;

where the first capture probe has a first adapter segment sequence that is different from the first adapter segment sequence of the second capture probe; and

where the first capture probe has an miRNA binding segment sequence that is different from the miRNA binding segment sequence of the second capture probe.

20 53. The method of claim 33, where the capture probe provided is a set of capture probes;

where the set comprises a first capture probe and a second capture probe;

where the first capture probe has a first adapter segment sequence that is different from the first adapter segment sequence of the second capture probe;

25 where the first capture probe has an miRNA binding segment sequence that is different from the miRNA binding segment sequence of the second capture probe; and

where the first capture probe has an miRNA binding segment sequence that is different from the miRNA binding segment sequence of the second capture probe.

30 54. The method of claim 33, where the capture probe provided is a set of capture probes;

where the set comprises a first capture probe having a first capture probe sequence, a second capture probe having a second capture probe sequence; and a third capture probe

having a third capture probe sequence;

where the first capture probe sequence is different from the second capture probe sequence;

where the first capture probe sequence is different from the third capture probe sequence; and

where second capture probe sequence is different from the third capture probe sequence.

55. The method of claim 33, where the first linker segment and the second linker segment comprise a substance selected from the group consisting of one or more than one type of polynucleotide, one or more than one type of polynucleotide analog, and a combination of one or more than one type of polynucleotide and polynucleotide analog.

56. The method of claim 33, where the first linker, or the second linker, or both the first linker and the second linker are resistant to nuclease degradation.

57. The method of claim 56, where the first linker, or the second linker, or both the first linker and the second linker comprise nuclease resistant nucleotides.

58. The method of claim 56, where the first linker, or the second linker, or both the first linker and the second linker comprise nucleotides with a phosphothioate backbone that render the first linker, or the second linker, or both the first linker and the second linker resistant to nuclease degradation.

59. The method of claim 56, where the first linker, or the second linker, or both the first linker and the second linker comprise nuclease resistant nucleotides and comprise nucleotides with a phosphothioate backbone that render the first linker, or the second linker, or both the first linker and the second linker resistant to nuclease degradation.

60. The method of claim 33, where the first linker and the second linker, each comprises between 6 and 50 residues.

61. The method of claim 33, where the first linker comprises at least 10 residues, and at least 10 residues at the 3' end of the first linker are exactly the complement of the corresponding residues at or near the 5' end of the first adapter segment.

62. The method of claim 33, where the second linker comprises at least 10 residues, and at least 10 residues at the 5' end of the second linker are exactly the complement of the corresponding residues at or near the 3' end of the second adapter segment.

63. The method of claim 33, where the 5' end of the first linker, or the 3' end of the

second linker, or both the 5' end of the first linker and the 3' end of the second linker comprise a label.

5 64. The method of claim 33, where the 5' end of first linker comprises one or more than one residue that extends beyond the 3' end of the first adapter segment after the first linker hybridizes to the first adapter segment.

65. The method of claim 64, where the one or more than one residue of the 5' end of first linker that extends beyond the 3' end of the first adapter segment functions as a primer binding site.

10 66. The method of claim 33, where the 3' end of second linker comprises one or more than one residue that extends beyond the 5' end of the second adapter segment after the second linker hybridizes to the second adapter segment.

67. The method of claim 66, where the one or more than one residue of the 3' end of second linker that extends beyond the 5' end of the second adapter segment functions as a primer binding site.

15 68. The method of claim 33, where the sample, the capture probe, the first linker and the second linker are combined simultaneously.

69. The method of claim 33, further comprising adding one or more than one RNase inhibitor to the combination of the sample, the capture probe, the first linker and the second linker.

20 70. The method of claim 33, where the first adapter segment comprises a solid phase binding group, or the second adapter segment comprises a solid phase binding group, or both the first adapter segment comprises a solid phase binding group and the second adapter segment comprises a solid phase binding group; and

25 where the method further comprises binding the capture probe to a solid phase before or after combining the sample, the capture probe, the first linker and the second linker.

71. The method of claim 70, where the solid phase is a plurality of paramagnetic particles.

30 72. The method of claim 70, where the capture probe is bound to a solid phase through the first adapter segment or through the second adapter segment or through both the first adapter segment and the second adapter segment; and

where the method further comprises purifying the capture probes with hybridized first linker, miRNA of interest and second linker--bound to the solid phase by removing

non-hybridized first linkers, second linkers and any other substances that are not bound to the solid phase.

73. The method of claim 70, where the solid phase is contained in a vessel comprising a surface and a cap, and where purifying comprises applying a magnetic field to attract the solid phase to the surface of the vessel or the cap of the vessel.

74. The method of claim 33, where the first linker hybridizes to the first adapter segment at a position where the last residue on the 3' end of the first linker hybridizes to a residue on the first adapter segment that is between 1 residue and 5 residues from the 3' end of the miRNA binding segment.

75. The method of claim 33, where the first linker hybridizes to the first adapter segment at a position where the last residue on the 3' end of the first linker hybridizes to a residue on the first adapter segment that is immediately adjacent to the 3' end of the miRNA binding segment.

76. The method of claim 33, where the second linker hybridizes to the second adapter segment at a position where the last residue on the 5' end of the second linker hybridizes to a residue on the second adapter segment that is between 1 residue and 5 residues from the 5' end of the miRNA binding segment.

77. The method of claim 33, where the second linker hybridizes to the second adapter segment at a position where the last residue on the 5' end of the second linker hybridizes to a residue on the second adapter segment that is immediately adjacent to the 5' end of the miRNA binding segment.

78. The method of claim 33, where the method further comprises purifying the complex.

79. The method of claim 33, where the complex is bound to a solid phase through the first adapter segment or through the second adapter segment or through both the first adapter segment and the second adapter segment; and

where the method further comprises purifying the complex by removing non-hybridized first linkers, second linkers and any other substances that are not bound to the solid phase.

80. The method of claim 33, where the method further comprises purifying the ligated first linker-miRNA of interest-second linker that has been dehybridized from the capture probe.

81. The method of claim 80, where the first linker, or the second linker, or both the first linker and the second linker comprise nuclease resistant nucleotides, or comprise nucleotides with a phosphothioate backbone that render the first linker, or the second linker, or both the first linker and the second linker resistant to nuclease degradation; and

5 where purifying the ligated first linker-miRNA of interest-second linker comprises applying DNAase to a solution containing the ligated first linker-miRNA of interest-second linker to destroy any DNA present in the solution.

82. The method of claim 80, where purifying the ligated first linker-miRNA of interest-second linker comprises circularizing the ligated first linker-miRNA of
10 interest-second linker.

83. A method for identifying an miRNA of interest, the method comprising:

a) isolating the miRNAs according to claim 33; and

b) sequencing the miRNA of interest portion of the strand of the ligated first linker-miRNA of interest-second linker.

15 84. The method of claim 83, where sequencing comprises subjecting the strand of the ligated first linker-miRNA of interest-second linker to reverse transcription to produce a double stranded product comprising a first strand of the ligated first linker-miRNA of interest-second linker and a second strand that is the complement of the first strand.

85. The method of claim 83, where sequencing comprises amplifying the double
20 stranded product to produce amplification products.

86. The method of claim 84, where sequencing comprises cloning the amplification products and culturing the amplification products.